

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

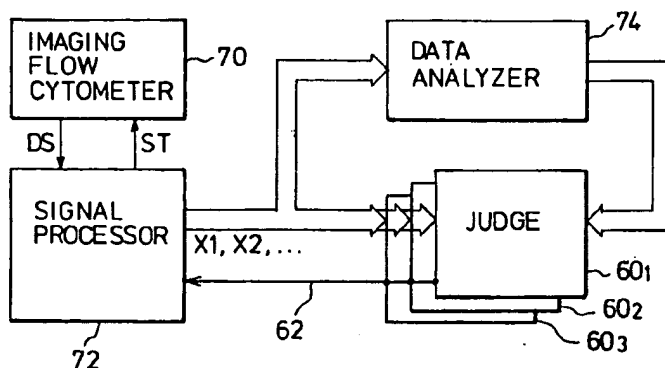
0 553 951 A1

(12)

EUROPEAN PATENT APPLICATION(21) Application number: **93300046.5**(51) Int. Cl.⁵: **G01N 15/14**(22) Date of filing: **06.01.93**(30) Priority: **30.01.92 JP 42319/92**(43) Date of publication of application:
04.08.93 Bulletin 93/31(84) Designated Contracting States:
DE FR GB IT(71) Applicant: **TOA MEDICAL ELECTRONICS
COMPANY, LIMITED**
**7-2-1, Minatojima-nakamachi, Chuoh-ku
Kobe-shi, Hyogo-ken(JP)**(72) Inventor: **Kosaka, Tokihiro**
**462-81, Ishimori, Kanno-cho
Kakogawa-shi, Hyogo-ken(JP)**(74) Representative: **Crawford, Andrew Birkby et al**
A.A. THORNTON & CO. Northumberland
House 303-306 High Holborn
London WC1V 7LE (GB)(54) **Particle judging device.**

(57) A device for identifying the kind of each particle detected by a particle detecting device such as imaging flow cytometer from detection signals obtained by passing therethrough a specimen, such as blood, in which various kinds of particles (blood corpuscles) exist intermixedly. Distribution characteristics (mean value, distribution width, etc.) of specific characteristic values previously sought on a known kind of reference particles are stored, the degree of similarity of the detected particle to the reference particles, that is, "probability of correctness of judge-

ment at the time of judging the detected particle as of the same kind of the reference particles" is calculated in accordance with a predetermined rule from the detection signals of the detected particle representing the same characteristic values and the stored distribution characteristics of the reference particles, and the probabilities of calculated on the respective characteristic values are synthesized to judge whether the detected particle is of the same kind as the reference particles or not.

**FIG. 7****EP 0 553 951 A1**

BACKGROUND OF THE INVENTION

This invention relates to a device for identifying the kind of each particle based upon detection signals obtained by passing a specimen such as blood, in which plural kinds of particles such as blood corpuscles exist intermixedly, through a particle detecting device such as flow cytometer.

With a flow cytometer, it is possible to catch each blood corpuscle in the blood and count the number of blood corpuscles and, also, to obtain various detection signals corresponding to each blood corpuscle, which suggest the kind thereof, by applying predetermined preliminary treatment to the blood. The detection signals are quantitative representation of various physical quantities (hereinafter referred to as "characteristic parameters") such as scattered light intensity, fluorescence intensity, light absorption, particle diameter, particle area and N/C ratio (the ratio of nucleus and cytoplasm when the particle is formed thereof) which suggest the kind of corresponding particle. Some methods and devices for identifying the kind of blood corpuscle using such detection signals have been known already as disclosed in U.S. Patent Nos. 3,883, 247 and 4,596,035, for example. According to these prior art techniques, however, sub-populations or "clusters" formed of respective kinds of blood corpuscles are sought from a frequency distribution diagram or histogram, or a correlative distribution diagram of the detection signals of a substantial number of particles to know quantitative proportion thereof, and it is impossible to know the kind of each blood corpuscle which is passing through a detecting area of the flow cytometer in real time fashion. It should be epoch-makingly advantageous for speeding up quantitative analysis of particle mixture such as blood, if the kind of each particle passing through the detecting area of the particle detecting device such as flow cytometer could be identified in real time fashion. However, only the following system has been proposed as what enables it. This system is based upon such a principle in that the value of each characteristic parameter substantially falls within a specific range relating to the kind of particle.

As shown in FIG. 1, detection signals X1 and X2 representing suitable two of the above characteristic parameters are selected and their upper limits X1max and X2max and lower limits X1min and X2min, which are experientially selected for a specific kind of particle, are stored in upper limit registers 10 and 12 and lower limit registers 14 and 16, respectively. The detection signals X1 and X2 are supplied respectively to a pair of comparators 18 and 20 and another pair of comparators 22 and 24 and compared with the contents of the upper

and lower limit registers 10 and 14 and the upper and lower limit registers 12 and 16. The comparators 18 and 20 deliver "high" level signals to an AND gate 26 when the signal X1 is within the prescribed range and, similarly, the comparators 22 and 24 deliver "high" level signals to an AND gate 28 when the signal X2 is within the prescribed range. The outputs of both AND gates are supplied to an AND gate 30 and, therefore, the AND gate 30 delivers a "high" level output when both detection signals are within the prescribed ranges, respectively. When the output signal of the AND gate 30 is of "high" level, it is judged that the particle which provides these detection signals shall belong to the specific kind. Although, two kinds of characteristic parameters are used in FIG. 1, three or more kinds of characteristic parameters may be used and the accuracy of judgement will rise with the number of kinds. As is obvious from FIG. 1, however, it is judged that the particle does not belong to the specific kind in this logic circuit, if even one of the outputs of the comparators 18, 20, ... is of "low" level. Therefore, there is a substantial chance of judging "true" as "false". However, if the prescribed range is expanded for removing the above problem, the probability of judging "false" as "true" will rise to reduce the accuracy of judgement.

As described above, the prior art technique could not obtain accurate judgement without mistake and, moreover, it could not numerically indicate the degree of certainty, or the probability of correctness, of each judgement.

Accordingly, an object of this invention is to provide an improved particle judging device which can identify the kind of each particle caught by a particle detecting device at high accuracy in real time fashion and, also, numerically indicate the probability of correctness of the judgement.

SUMMARY OF THE INVENTION

The above object is achieved in accordance with this invention which provides a particle judging device comprising means for providing a detection signal representing at least one characteristic parameter of each particle, means for storing distribution characteristics of the same characteristic parameter which are previously sought on a specific kind of particles, data converting means for converting the detection signal based upon these distribution characteristics in accordance with a predetermined rule to calculate the probability of each particle being a particle of the specific kind, and means for comparing the calculated probability with a predetermined reference value to judge whether the particle belongs to the specific kind or not.

In a preferred example, the stored distribution characteristics are a mean value and a distribution width, and the data converting means includes means for calculating the deviation of the detection signal from the mean value, means for normalizing the deviation based upon the distribution width and means for converting the normalized deviation in accordance with a predetermined function.

Now, these and other features and functions of this invention will be described in more detail below about a preferred embodiment thereof with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

FIG. 1 is a block diagram showing a configuration of a particle judging device according to prior art, which is a start point of this invention;

FIG. 2 is a block diagram showing an overall configuration of a preferred embodiment of the particle judging device according to this invention;

FIG. 3 is a block diagram showing a schematic configuration of a data converting unit in the embodiment of FIG. 2;

FIGS. 4a to 4d are diagrams showing examples of the function used in the data converting unit of FIG. 3;

FIG. 5 is a block diagram showing a schematic configuration of the data converting unit in the embodiment of FIG. 2;

FIG. 6 is a block diagram showing a schematic configuration of a variation of the embodiment of FIG. 2; and

FIG. 7 is a block diagram showing the embodiment of FIG. 2 co-operated with an imaging flow cytometer.

Throughout the drawings, same reference numerals are given to the same structural components.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring to FIG. 2, the particle judging device of this invention includes converting units 32₁, 32₂, ... which receive detection signals X₁, X₂, ... representing characteristic parameters of each particle, which are supplied from a particle detecting device (not shown) such as flow cytometer, and a judging unit 34 which receive the outputs of these converting units. The converting units 32₁, 32₂, ... respectively store probability distribution functions $Y_1 = f_1(X_1)$, $Y_2 = f_2(X_2)$, ... of detection signals representing the same characteristic parameters, which are previously sought on a specific kind of particles. Therefore, these function values Y₁, Y₂,

... represent the frequency of appearance of the detection signals when the detected signal belongs to the specific kind, that is, the probability of the detected particle belonging to the specific kind at the time of detection signals X₁, X₂, ..., respectively. The input detection signals X₁, X₂, ... are converted respectively into the values Y₁, Y₂, ... representing the probabilities in accordance with the above-mentioned functions and, also, weighted respectively with separately provided weighting coefficients a₁, a₂, ... in the converting units 32₁, 32₂, ..., respectively, and the resultant weighted outputs $Ya_1 (= a_1 \cdot Y_1)$, $Ya_2 (= a_2 \cdot Y_2)$, ... are supplied to the judging unit 34, as shown. The coefficients a₁, a₂, ... are constants from zero to one, which are determined in accordance with the judging rule as described below. The judging unit 34 synthesizes the input signals Ya₁, Ya₂, ... to calculate a value indicative of the synthesized probability and, also, compares it with a predetermined value to provide a judgement signal Z₁. The processing in the converting units 32₁, 32₂, ... and judging unit 34 are effected in real time fashion and, in fact, the time needed from reception of the detection signals X₁, X₂, ... to delivery of the judgement signal is less than several hundred nanoseconds.

As shown in FIG. 3, the converting unit 32₁ includes a mean value register 36 for storing a mean value U₁ of the distribution expressed by the above function $f_1(X_1)$, a distribution width register 38 for storing its distribution width W₁ and a subtracter 40 and a normalizing unit 42 to which the contents of the registers 36 and 38 are supplied respectively. The distribution width may be selected as three times the standard deviation, for example. The subtracter 40 subtracts the mean value U₁ from the input X₁ to provide an absolute deviation $|X_1 - U_1|$, and the normalizing unit 42 calculates a normalized deviation ΔX_1 from the absolute deviation and the distribution width W₁ in accordance with the following equation.

$$\Delta X_1 = k_1 (|X_1 - U_1| / W_1)$$

where k₁ is a constant.

The normalized deviation ΔX_1 is supplied to a function table memory 44 which is provided with a function select register 46 for selecting a function $f_1(\Delta X_1)$. The function f_1 represents the relation between the normalized deviation ΔX_1 and the probability of the corresponding detection particle being a particle of the specific kind and it is determined on the basis of frequency distribution data of each characteristic parameter of the specific kind of particles and likes. While this function is a decremental function whose value is maximum at zero value of the normalized deviation ΔX_1 and becomes zero at a certain value k thereof, as

shown in FIGS. 4a to 4c, it is possible to show the probability of the detected particle being not a particle of the specific kind with the value of the function if it is such an incremental function as shown in FIG. 4d. The normal distribution curve of FIG. 4c is suitable as the function f_1 since the frequency distribution curve of the characteristic parameter value is generally a normal distribution curve. However, such an approximate linear function as shown in FIG. 4a or 4b may be used for convenience in the function select register 46. One select code of such functions is preset by an external device (not shown) and supplied to the function table memory 44, in which its value $Y1 = f_1(\Delta X1)$ is calculated.

The function table memory 44 is further provided with a weighting coefficient register 48 for storing various values of the weighting coefficient a_1 . The value of the weighting coefficient a_1 , which is specified in accordance with the undermentioned judging rule, is preset by an external device (not shown) and supplied to the function table memory 44. The table memory 44 calculates $Ya_1 = a_1 \cdot Y1$ to deliver the probability output Ya_1 .

It is recommendable to use a look-up table system for arithmetic operation of the units 40, 42 and 44 in order to raise their operation speed to effect real time indication. Although the other converting units 32₂, ... of FIG. 2 may have the same circuit configuration as the above-mentioned converting unit 32₁, it is necessary to select the probability functions f_2 , ... and weighting coefficients a_2 , ... so as to conform to the corresponding characteristic parameters, respectively.

Referring next to FIG. 5, the outputs Ya_1 , Ya_2 , ... of the converting units 32₁, 32₂, ... are supplied to an adder circuit 50 in the judging unit 34 and summed up and a resultant sum $T (= Ya_1 + Ya_2 + \dots)$ is supplied as an object value of judgement to a comparator, which is provided with a reference register 52 for storing a reference value S for judgement, and compared with the reference value S . The comparator 54 is arranged to provide a "high" level output when the object value T is higher than the reference value S and its output terminal is connected to one of the input terminals of an exclusive-NOR circuit 56. The other input terminal of the exclusive-NOR circuit 56 is grounded through a switch 58 and also connected to a "high" level source terminal H through a resistor 59 so as to selectively receive a "high" or "low" level input. Therefore, the output $Z1$ of the exclusive-NOR circuit 56 shows affirmative decision at "low" level when the switch 58 is closed as shown, while it shows the same decision at "high" level when the switch 58 is open.

The above-mentioned judging process is executed in accordance with such a rule as described

below in connection with some examples. In the following description, judgement is effected upon three characteristic parameters and detection signals $X1$, $X2$ and $X3$ respectively representing the values thereof are handled. For simplicity, however, the detection signals $X1$, $X2$ and $X3$ will be simply referred to as characteristic parameters $X1$, $X2$ and $X3$. Moreover, the particle which is caught by the particle detector and whose kind is to be identified will be referred to as "subject particle" and the particle of known kind, whose identity to the "subject particle" is to be judged, will be referred to as "object particle".

Rule-Example 1

A subject particle satisfying the following three conditions shall be judged as an object particle.

- (1) Its characteristic parameter $X1$ is approximate to the mean value of the characteristic parameter $X1$ of the object particle,
- (2) its characteristic parameter $X2$ is approximate to the mean value of the characteristic parameter $X2$ of the object particle, and
- (3) its characteristic parameter $X3$ is approximate to the mean value of the characteristic parameter $X3$ of the object particle.

When the subject particle is an object particle, the judgement output $Z1$ of the exclusive NOR circuit 56 shall be at "low" level and the above-mentioned three conditions shall be equivalent in significance.

In order to realize the above-mentioned rule, the values of weighting coefficients a_1 , a_2 , and a_3 are made equal to unify the three conditions in significance, when the mean values, distribution widths, distribution functions and weighting coefficients of the characteristic parameters of the object particle is set respectively in the registers 36, 38, 46 and 48 of the converting units 32₁, 32₂ and 32₃. The switch 58 of the judging unit is closed so that the output $Z1$ of the exclusive NOR circuit 56 is at "low" level in response to the "high" level output of the comparator 54. When there is such a relation as $T \geq S$ in this state between the sum T of the probability outputs Ya_1 , Ya_2 and Ya_3 of the respective converting units 32₁, 32₂ and 32₃ and the reference probability S , the output $Z1$ of the judging unit is at "low" level and the subject particle is judged as an object particle at probability T .

Rule-Example 2

A subject particle satisfying the following three conditions shall be judged as an object particle.

- (1) Its characteristic parameter $X1$ is approximate to the mean value of the characteristic parameter $X1$ of the object particle,

- (2) its characteristic parameter X2 is approximate to the mean value of the characteristic parameter X2 of the object particle, and
- (3) its characteristic parameter X3 is approximate to the mean value of the characteristic parameter X3 of the object particle.

When the subject particle is an object particle, the judgement output Z1 of the exclusive NOR circuit 56 shall be at "low" level and the above-mentioned three conditions shall be high, medium and low in significance in this order.

In order to realize the above-mentioned rule, while the respective registers are set as same as in the case of Example 1, the weighting coefficients a_1 , a_2 and a_3 are set in the weighting coefficient registers in the converting units 32₁, 32₂ and 32₃ so as to make their mutual relation $a_1 > a_2 > a_3$. Moreover, the switch 58 of the judging unit is closed so that the output Z1 of the exclusive NOR circuit 56 is at "low" level in response to the "high" level output of the comparator 54. When the probability outputs of the converting units 32₁, 32₂ and 32₃ are weighted differently as above, it is possible to effect more accurate judgement giving consideration to the significance of each condition. If the weighting coefficient is made zero, the corresponding characteristic parameter becomes to be disregarded.

It is also considered to apply plural rules differing in their contents as Examples 1 and 2 and provide synthesized affirmative decision in response to affirmative decision in accordance with any rule of them. In this case, as shown in FIG. 6, the outputs Z1, Z2, ... of particle judging blocks 60₁, 60₂, ... which execute their own rules respectively as above are connected in parallel by a wired OR line 62 and the output WZ of the wired OR line is connected to one input of an exclusive NOR circuit 64. As same as the exclusive NOR circuit 56, the other input of the exclusive NOR circuit 64 is grounded through a switch 66 and also connected through a resistor 68 to a "high" level source terminal H. The output WZ of the wired OR line 62 become "low" level if any one of the particle judging blocks 60₁, 60₂, ... (whose switches 58₁, 58₂, ... corresponding to the switch 58 are all closed) produces an affirmative output at "low" level. When the switch 64 is closed, therefore, the synthesized decision output Z of the exclusive NOR circuit 64 becomes "high" level if the condition based upon any judging rule is satisfied. When the switch 66 is open, the synthesized decision output Z becomes "high" level only when the outputs Z1, Z2, ... of all blocks 60₁, 60₂, ... are at "high" level, that is, when no condition based upon any rule is satisfied. Thus, it is possible to arbitrarily obtain affirmative or negative decision by controlling the switch 66.

The configuration of FIG. 6 has such an advantage in that the judging conditions can be easily changed, since the particle judging blocks 60₁, 60₂, ... storing various preset rules can be freely attached to and detached from the wired OR line.

FIG. 7 shows a particle identifying system including particle judging blocks 60₁, 60₂, ... as described above combined with an imaging flow cytometer 70 as a particle detecting device. In this system, it is also possible to pick up and display a still image of each particle. The imaging flow cytometer 70 includes flow cell mechanism for forming a flat sheath flow of specimen, a light source and a light sensor for detecting characteristics of each particle, a stroboscopic light source and a video camera for picking up the image of the particle, and likes. A detection signal DS of each particle obtained in the flow cytometer is supplied to a signal processing device 72 and converted here to respective parameter values X1, X2, ... in real time fashion. The characteristic parameter values X1, X2, ... are supplied respectively to particle judging blocks 60₁, 60₂, ... having various judging rules preset therein. The judging blocks return their decision outputs (Z1, Z2, ...) through a wired OR line 62 to the signal processing device 72 within several hundred nanoseconds. The signal processing device 72 includes a circuit equivalent to the exclusive NOR circuit 64 and, when the subject particle is judged as an object particle, delivers a stroboscopic triggering signal ST to the flow cytometer 70. The stroboscopic light source of the flow cytometer flashes in response to the signal ST and the image of the subject particle is picked up by the video camera. The characteristic parameter values obtained from the signal processing device 72 are also supplied to a data analyzing device 74 and provided for statistical analysis using histograms and multidimensional scattergrams of the respective characteristic parameters. The result of analysis is supplied to the respective particle judging blocks 60₁, 60₂, ... for use in modification of the above-mentioned judging rules and preset conditions stored respectively therein.

The particle judging device of this invention can be combined with an imaging flow cytometer for picking up the image of subject particle and, moreover, it may be combined with a sorting device such as cell sorter for collecting the object particles only.

The above description of the embodiment is given only for the purpose of illustration and do not mean any limitation of the invention. It should be obvious to those skilled in the art that various modification and changes can be made on the above embodiment within the scope of the invention as defined in the appended claims.

Claims

1. A particle judging device including means for detecting each particle from a specimen in which plural kinds of particles exist intermixedly and producing a detection signal representing the value of at least one of characteristics of said particle, characterized in that said device further comprises:
 - means for storing distribution characteristics of said characteristic parameter, which is previously sought on a specific kind of particles,
 - data conversion means for converting said detection signal based upon said distribution characteristic in accordance with a predetermined rule to calculate probability of said particle belonging to said specific kind, and
 - means for judging whether said particle belongs to said specific kind or not, on the basis of said probability calculated by said data conversion means.
2. A particle judging device as set forth in claim 1, in the case of using detection signals representing the values of plural characteristic parameters of each particle, characterized in that said data conversion means includes means for weighting the respective probabilities derived from said detection signals.
3. A particle judging device as set forth in claim 1, in the case of using detection signals representing the values of plural characteristic parameters of each particle, characterized in that said data conversion means includes means for calculating the probabilities derived from said detection signals.
4. A particle judging means as set forth in claim 1, characterized in that said data conversion means includes means for storing a mean value and a distribution width of said characteristic parameter of said specific kind of particles, means for calculating deviation of the value of said characteristic parameter represented by said detection signal from said mean value, means for normalizing said deviation based upon said distribution width to obtain normalized deviation, means for storing a predetermined probability distribution function, and means for seeking probability of said normalized deviation based upon said probability distribution function.
5. A particle judging device as set forth in claim 4, characterized in that said function storing means includes means for storing plural functions and selecting one of said functions and supplying the same to said probability seeking means.
6. A particle judging device as set forth in claim 4, characterized in that said probability seeking means is provided with means for weighting the probability obtained therefrom.
7. A particle judging device as set forth in claim 1, characterized in that said device further comprises switch means for selectively inverting a logic of the output of said judging means.
8. A particle judging device as set forth in claim 3, characterized in that said device comprises a plurality of judging means corresponding to a plurality of particle judging rules, and wired OR means having the results of judgement of said judging means for delivering a judgement signal at the output thereof.

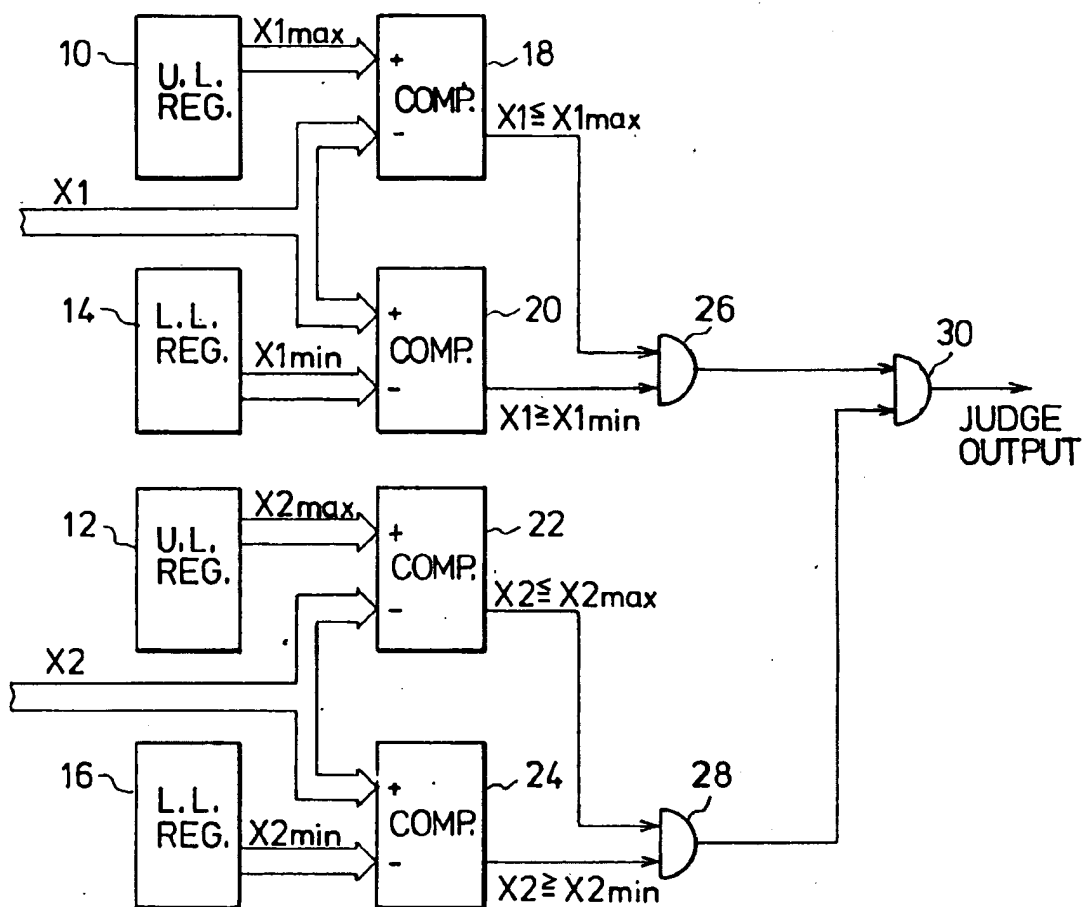


FIG. 1 (PRIOR ART)

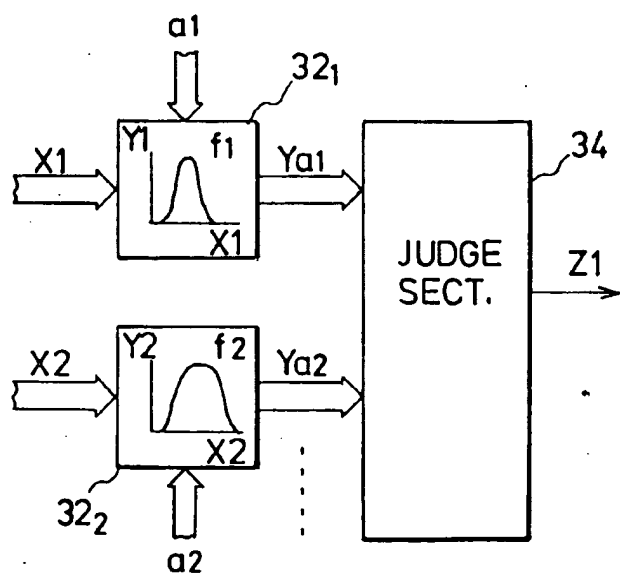


FIG. 2

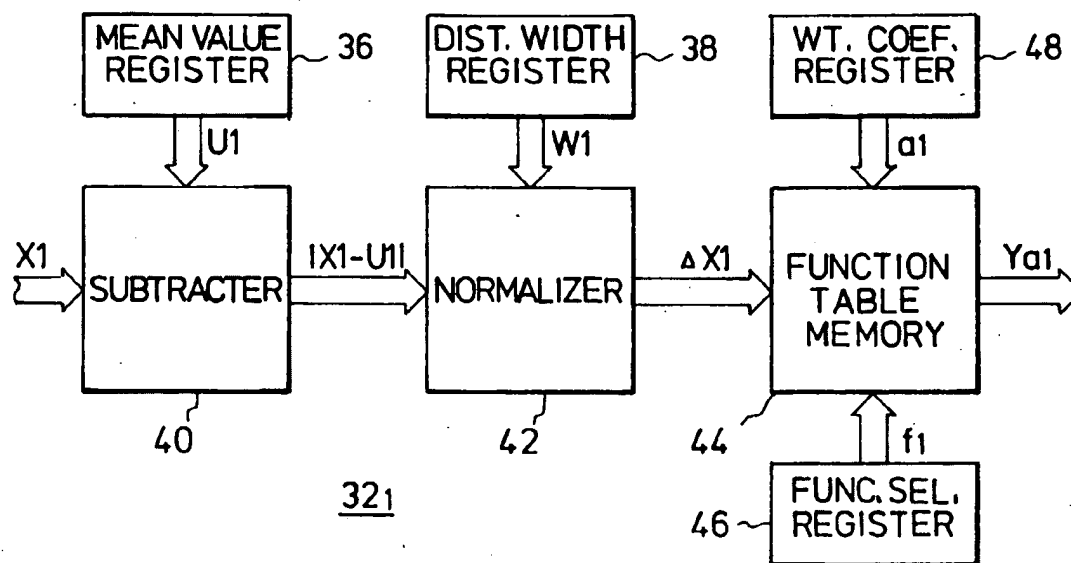


FIG. 3

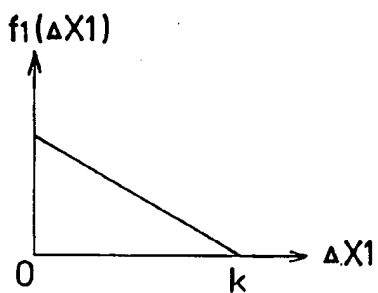


FIG. 4a

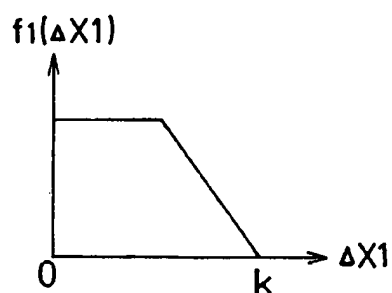


FIG. 4b

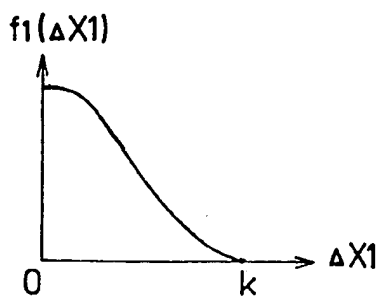


FIG. 4c

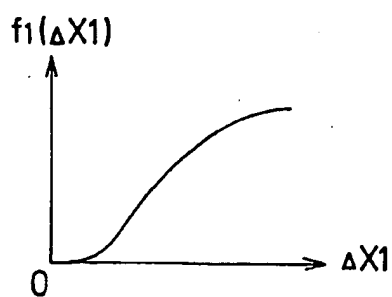


FIG. 4d

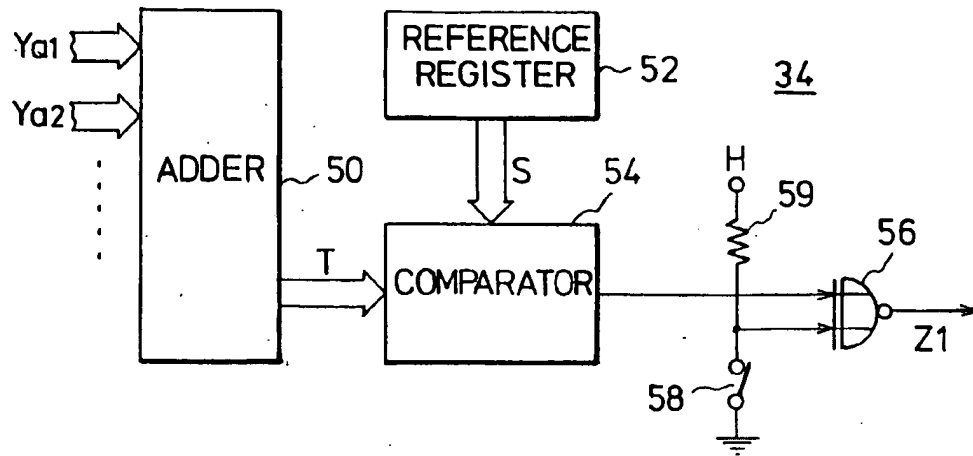


FIG. 5

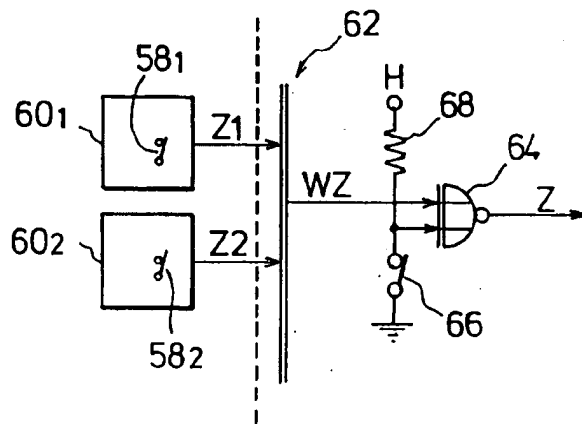


FIG. 6

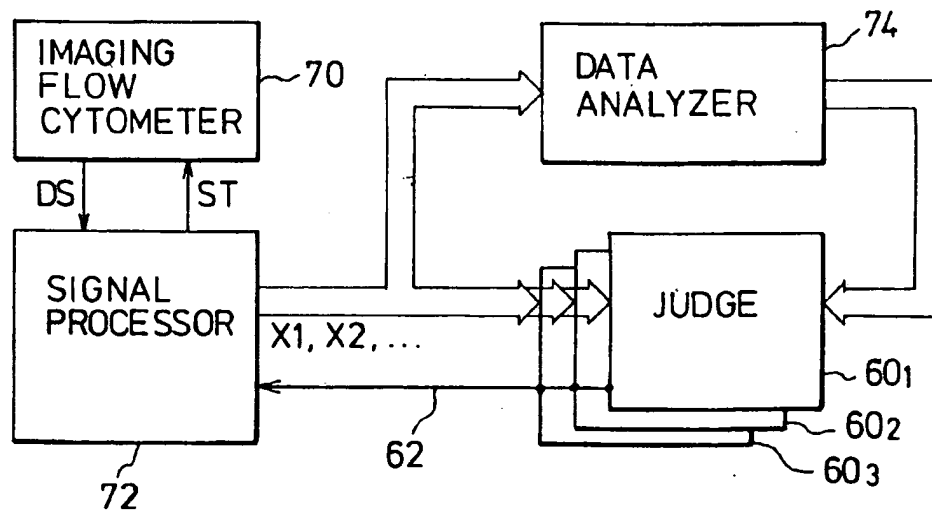


FIG. 7



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 93 30 0046

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 022 670 (ORTHO DIAGNOSTICS INC.) * abstract; figure 2 * ---	1	G01N15/14
A	EP-A-0 335 001 (TOA MEDICAL ELECTRONICS CO.) * the whole document * ---	1	
A	CA-A-1 233 250 (ROYAL VICTORIA HOSPITAL) * page 1 * ---	1	
A	US-A-3 826 364 (W. A. BONNER ET AL.) * figure 1A * ---	1	
A	US-A-4 661 913 (HAI-PING WU ET AL.) * abstract * ---	1	
A	US-A-5 041 733 (Y. NOGUCHI ET AL.) * abstract; figure 8 * ---	1	
A,P	US-A-5 117 357 (T. INOUE) * the whole document * -----	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G01N
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 13 MAY 1993	Examiner BRISON O.P.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ***** & : member of the same patent family, corresponding document			